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Response of the Pulmonary Circulation to Acetylcholine, Calcitonin Gene-Related Peptide, Substance P and Oral Nicardipine in Patients With Primary Pulmonary Hypertension

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Endothelium-dependent vasodilation of the pulmonary vascular bed was investigated in five patients with primary pulmonary hypertension. Three endothelium-dependent vasodilators (acetylcholine, calcitonin gene-related peptide and substance P [in two patients]) were infused sequentially into the right atrium, followed by nicardipine given orally during full hemodynamic monitoring.

Acetylcholine, calcitonin gene-related peptide and substance P had no effect on pulmonary artery pressure, total pulmonary vascular resistance or cardiac output, although calcitonin gene-related peptide significantly decreased systemic arterial systolic pressure from 132 ± 34 to 113 ± 33 mm Hg. In contrast, oral nicardipine decreased total pulmonary vascular resistance from 23 ± 12 to 13 ± 8 U, with a concomitant increase in cardiac output from 3.1 ± 1 to 4.7 ± 2 liters·min^{-1} and decrease in systemic vascular resistance from 39 ± 9 to 13 ± 4 U.

Thus, despite the presence of a reversible component in these five patients with primary pulmonary hypertension, pulmonary vascular resistance did not decrease in response to the infused endothelium-dependent vasodilator agents, indicating that endothelium-dependent vasodilation is impaired in these patients.

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Primary pulmonary hypertension is uncommon and its cause is unknown. It occurs most frequently in women, particularly in the 3rd and 4th decades, and the mean survival time after symptom onset is only 2 to 3 years (1). Early studies suggested that arteriolar vasoconstriction was a major component of the increased pulmonary artery pressure; a reduction in pulmonary artery pressure in response to intravenous aminophylline and tolazoline (2) and intrapulmonary infusion of acetylcholine (3) was reported in patients with pulmonary hypertension. One early histopathologic series (4) showed that in the majority of cases of primary pulmonary hypertension, medial hypertrophy of the small pulmonary arteries and "plexogenic" lesions occur and this was interpreted as evidence for a vasoconstrictive etiology. The proposal that the condition was due to pulmonary vasoconstriction in response to an external stimulus (4,5) prompted many nonrandomized studies (6–9) using different vasodilator drugs. Unfortunately, treatment with vasodilators has not been shown to improve survival (9), despite a variable short-term effect on pulmonary artery pressure and pulmonary vascular resistance (8–10).

Recently, interest has focused on the pulmonary endothelial cells (11,12) and it has been proposed that the plexogenic arteriopathy, which is found in up to 50% of cases (13), may result from endothelial cell injury and this may affect endothelium-derived mediators of smooth muscle tone (11). It is possible that part of the increased pulmonary vascular resistance in these patients is due to impaired endothelium-dependent vasodilation. This study was designed to investigate the hemodynamic response of the pulmonary circulation to the vasoactive agents acetylcholine (an endothelium-dependent vasodilator and smooth muscle constrictor [14]), substance P (an endothelium-dependent vasodilator [14]) and calcitonin gene-related peptide (an endothelium-dependent and endothelium-independent vasodilator [15]). This response was compared with that after cumulative oral dosing with the dihydropyridine calcium channel blocker and smooth muscle dilator nicardipine.

Methods

Study patients (Table 1). Five consecutive patients with unexplained pulmonary hypertension were studied. Their clinical and baseline hemodynamic characteristics are shown in Table 1. A diagnosis of primary pulmonary hypertension had been made after exclusion of other cardiac and respiratory causes of pulmonary hypertension.

All patients were admitted to the coronary care unit and written consent for the procedures was obtained in each instance. The protocol was approved by the Research Ethics Committee of Hammersmith Hospital.

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Table 1. Clinical and Hemodynamic Characteristics of the Five Study Patients

Pt No.	Age (yr)/ Gender	Presenting Symptoms	Duration From Symptom Onset (mo)	SAP (mm/Hg)	PAP (mm/Hg)	PAP_{mean} (mm/Hg)	$PAWP_{mean}$ (mm/Hg)	TPVR (U)	Cardiac Output (liters·min^{-1})	Follow-Up (mo)	Present Status
1	30/F	Dyspnea, lethargy	80	140/60	97/55	68	11	13.0	5.3	24	Stable
2	38/M	Dyspnea, edema	42	96/60	74/24	41	5	15.9	2.6	2	Dead
3	45/F	Chest pain, dyspnea	36	125/77	117/49	75	3	40.1	1.9	8	Dead
4	32/F	Dyspnea	6	110/58	82/36	50	11	11.5	4.4	8	Stable
5	33/F	Chest pain, dyspnea	24	148/78	88/27	52	0	14.8	3.5	6	Improved
Mean	36					57	6	19.1	3.5		
SEM	3					6	2	5.3	0.6		

F = female; M = male; PAP = pulmonary artery pressure; PAWP = pulmonary artery wedge pressure; Pt = patient; SAP = systemic arterial pressure; TPVR = total pulmonary vascular resistance.

Measurements. A triple-lumen thermodilution catheter (7F gauge, American Edwards Laboratories) was positioned in a branch of the right pulmonary artery to allow measurement of right atrial, pulmonary artery and pulmonary artery capillary wedge pressures. A left radial artery catheter (Abbocath-T, 20 gauge; Abbott Laboratories) or right femoral artery catheter (7F gauge; Cordis UK Ltd.) was inserted to allow measurement of systemic artery pressure. Cardiac output was determined by the indirect Fick method; oxygen saturation was measured in samples of pulmonary artery and systemic artery blood. Total pulmonary vascular resistance was calculated from the mean pulmonary artery pressure divided by the cardiac output. Systemic vascular resistance was calculated from the difference between the mean systemic arterial pressure and mean right atrial pressure and divided by the cardiac output. Both total pulmonary and systemic vascular resistance were expressed as arbitrary resistance units (dynes·s·cm^{-5}/80). Heart rate was determined by continuous electrocardiographic monitoring and pulmonary and systemic arterial pressures were displayed continuously on a bedside monitor (Marquette Electronics).

Protocol. Pulmonary and systemic arterial pressures were recorded over 60 min to ensure that the baseline values were stable. Serial determinations of right atrial, pulmonary and systemic arterial pressures, heart rate and cardiac output were obtained at 5-min intervals. The administration of any agent was discontinued if systemic arterial pressure decreased by >30 mm Hg or if any symptoms developed.

Acetylcholine (Sigma Laboratories) was infused in incremental doses to give estimated pulmonary artery blood concentrations of 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} *M*. Each dose was given for 5 min into the right atrium through the proximal lumen of the catheter. We selected this dosage regimen because progressive vasodilation occurs in normal coronary arteries during acetylcholine infusion with estimated blood concentrations of 10^{-8} to 10^{-6} *M* (16,17) and higher concentrations are associated with vasoconstriction (16). After a 30-min interval to allow the variables to return to baseline values, calcitonin gene-related peptide (Sigma Laboratories) was infused at doses of 10, 50, 100, 150 and 200 pmol·min^{-1}, each dose for 5 minutes, and serial measurements were again determined.

The first two patients studied also received incremental intracardiac infusions of substance P (Sigma Laboratories), each dose over 5-min intervals. This was given at doses of 1 and 5 pmol·min^{-1} in Patient 1 and at 1, 5, 10, 15, 20 and 35 pmol·min^{-1} in Patient 2. In both patients, marked symptomatic flushing and nausea occurred, although there was no change in systemic or pulmonary artery pressure or cardiac output. In view of this, substance P infusions were not given to the subsequent three patients. One hour after discontinuation of the calcitonin gene-related peptide infusion, patients received 20 mg of oral nicardipine, every hour up to a maximal dose of 200 mg or until systemic arterial pressure decreased by >30 mm Hg or symptoms developed.

Because of the tendency toward spontaneous hemodynamic variability in primary pulmonary hypertension (18), we defined a decrease in pulmonary artery pressure of 10% accompanied by a reduction in total pulmonary vascular resistance of ≥30% as a significant response, as previously suggested by Palevsky et al. (19).

Statistical analysis. Data were analyzed with use of the Student paired *t* test. All values are expressed as mean values ±1 SEM; a value $p < 0.05$ was considered statistically significant.

Results

Acetylcholine (Table 2). Because acetylcholine causes vasodilation at low doses and vasoconstriction at higher doses in other vascular beds, we considered only the maximal decrease in total pulmonary vascular resistance, irrespective of the corresponding estimated pulmonary artery blood concentration, when determining the mean hemodynamic values. Thus, there was a small maximal decrease in total pulmonary vascular resistance (19 ± 5 to 15 ± 5 U, $p < 0.05$) during infusion of acetylcholine, but in individual patients, the maximal decrease in total pulmonary vascular resistance occurred at different estimated pulmonary artery blood concentrations (10^{-8} to 10^{-4} *M*) of acetylcholine (Fig.

Table 2. Effects of Acetylcholine and Calcitonin Gene-Related Peptide Infusion and Oral Nicardipine Administration in Five Patients

	Acetylcholine		Calcitonin Gene-Related Peptide		Nicardipine	
	Basal	10^{-8} to 10^{-5} M	Basal	100 to 200 pmol·min^{-1}	Basal	100 to 200 mg
Heart rate (min^{-1})	90 ± 10	97 ± 12	96 ± 8	103 ± 8*	95 ± 10	97 ± 8
Cardiac output (liters·min^{-1})	3.4 ± 0.5	4.5 ± 1.0	3.8 ± 0.8	4.3 ± 0.9	3.1 ± 0.4	4.7 ± 0.9*
PAP_{sys} (mm Hg)	91 ± 7	90 ± 11	95 ± 8	99 ± 12	102 ± 11	82 ± 10
SAP_{sys} (mm Hg)	121 ± 8	112 ± 12	132 ± 15	113 ± 15*	134 ± 14	99 ± 12*
TPVR (U)	19 ± 5	15 ± 5*	19 ± 5	17 ± 4	23 ± 5	13 ± 4*
SVR (U)	27 ± 6	18 ± 5*	26 ± 5	30 ± 2	30 ± 4	13 ± 2*
TPVR/SVR	0.70 ± 0.06	0.83 ± 0.12	0.71 ± 0.05	0.58 ± 0.10	0.76 ± 0.07	0.98 ± 0.14

*$p < 0.05$. PAP_{sys} = pulmonary artery systolic pressure; SAP_{sys} = systemic arterial systolic pressure; SVR = systemic vascular resistance; TPVR = total pulmonary vascular resistance.

1). However, the mean decrease in total pulmonary vascular resistance was only 21.5% (range 10.5% to 40.4%) and only in Patient 2 did it exceed the accepted criterion of 30%. In addition, inspection of Figure 1 shows not only that the decrease in total pulmonary vascular resistance during acetylcholine infusion was small, but also that it was not dose-related as in the coronary circulation. The cause of this variation in pulmonary vascular resistance is uncertain but may be due to the spontaneous variations in pulmonary hemodynamics that have been reported in pulmonary hypertension (18).

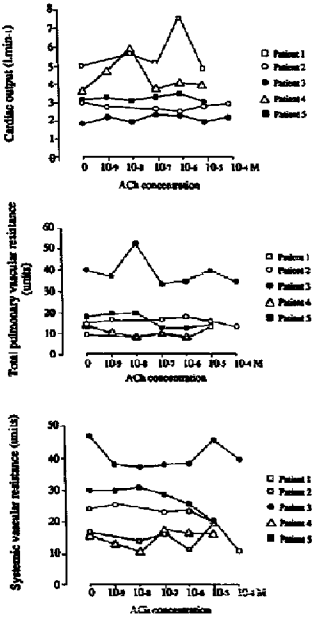

Figure 1. Individual responses of cardiac output, total pulmonary vascular resistance and systemic vascular resistance to increasing molar concentrations of acetylcholine (ACh) in five patients.

In two of the five patients (Patients 2 and 5), a reduction in pulmonary artery systolic pressure occurred (82 to 55 and 109 to 95 mm Hg, respectively), although in the other three it remained unchanged (Table 2). Maximal decreases in systemic vascular resistance were observed in individual patients again at different estimated pulmonary artery blood concentrations (10^{-8} to 10^{-4} *M*) of acetylcholine (mean decrease in systemic vascular resistance, 27 ± 6 to 18 ± 5 U, $p < 0.05$) (Fig. 1), but systemic arterial systolic pressure decreased only in Patients 2 and 5 (111 to 67 and 142 to 109 mm Hg, respectively).

Calcitonin gene-related peptide (Table 2). Calcitonin gene-related peptide did not affect mean total pulmonary vascular resistance, and only in Patients 3 and 4 was even a small decrease observed (Fig. 2). Pulmonary artery systolic pressure decreased (from 114 to 87 mm Hg) in Patient 1. Although there was a significant reduction in systemic arterial systolic pressure, the decrease in systemic vascular resistance in all five patients did not reach statistical significance. However, systemic vascular resistance decreased substantially in Patients 3 and 5 (59% and 40%, respectively) (Fig. 2).

Substance P. Substance P was given to only two patients and in both it caused severe flushing and nausea. Total pulmonary and systemic vascular resistance and pulmonary and systemic artery systolic pressure were unchanged in Patient 1 after the second incremental dose of 5 pmol·min^{-1} and in Patient 2 after a maximal dose of 35 pmol·min^{-1}.

Nicardipine (Table 2). Nicardipine significantly reduced total pulmonary vascular resistance and increased cardiac output in all five patients (Fig. 3). There was a reduction in pulmonary artery systolic pressure in Patients 1, 3 and 5 (from 101 to 63, 124 to 109 and 130 to 68 mm Hg, respectively). Systemic arterial systolic pressure and systemic

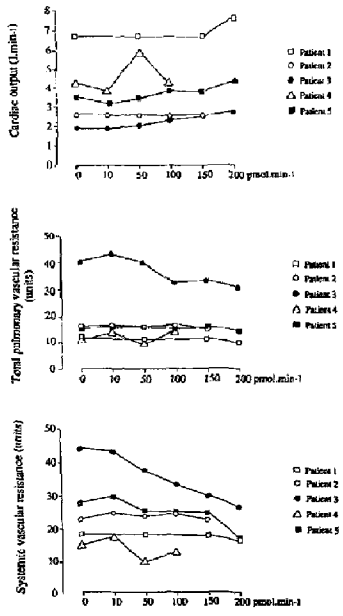

Figure 2. Individual responses of cardiac output, total pulmonary vascular resistance and systemic vascular resistance to increasing concentrations of calcitonin gene-related peptide.

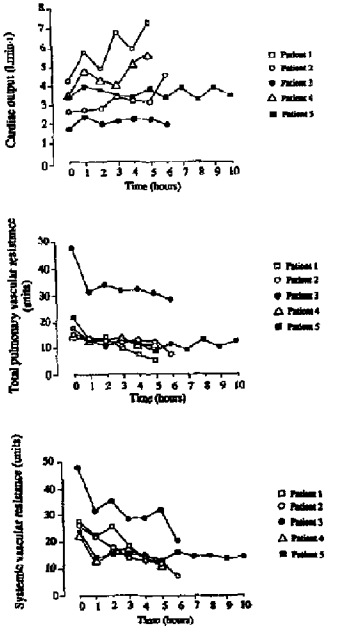

Figure 3. Individual responses of cardiac output, total pulmonary vascular resistance and systemic vascular resistance to cumulative dosing with oral nicardipine.

vascular resistance were decreased significantly; despite this, nicardipine was well tolerated in this short-term study.

Discussion

We used sequential infusions of acetylcholine, calcitonin gene-related peptide and substance P into the right atrium to investigate endothelium dependent vasodilation of the pulmonary vascular bed in five patients with primary pulmonary hypertension. In view of the spontaneous hemodynamic changes that can occur in pulmonary hypertension (18), we considered significant only those changes in total pulmonary vascular resistance >30% and pulmonary artery pressure >10% (19). According to these criteria, this study showed that acetylcholine and calcitonin gene-related peptide had no important effects on pulmonary vascular resistance in these five patients. Calcitonin gene-related peptide decreased systemic arterial systolic pressure in all patients and systemic vascular resistance in four. Similarly, substance P had no effect on pulmonary vascular resistance in the two patients who received it, although it produced flushing and nausea. In contrast, cumulative dosing with oral nicardipine reduced total pulmonary vascular resistance and systemic vascular resistance. These findings indicate that there is a vasoconstrictive component to the increase in pulmonary vascular resistance in these patients; this component can be decreased by vasodilators such as nicardipine, which act directly on vascular smooth muscle, but not by endothelium-dependent vasodilation.

Nicardipine is a dihydropyridine calcium antagonist that reduces contraction of cardiac and vascular smooth muscle

cells by competitively inhibiting calcium ion influx through the slow channel of plasma membranes. It is more selective for vascular smooth muscle than is nifedipine, diltiazem or verapamil (20). Because of high aqueous solubility, the peak plasma concentration of nicardipine is reached after 20 min to 2 h (21). Systemic bioavailability increases with increasing doses, suggesting saturation of first-pass metabolism, with an elimination half-life of approximately 1 h almost entirely due to hepatic metabolism (22). A previous study (22) indicated that after single oral doses of 10, 20, 30 and 40 mg, plasma concentrations of 13, 32, 91 and 253 $\mu g \cdot liter^{-1}$, respectively, are achieved (22).

Endothelium-dependent vasodilation of the pulmonary circulation. In normal subjects, pulmonary vasodilation occurs in response to intrapulmonary infusion of acetylcholine (23). In one study (23) of 13 patients, infusion of acetylcholine at a rate of 0.5 $mg \cdot min^{-1}$ resulted in a decrease in pulmonary artery pressure, with no change in pulmonary artery wedge pressure, systemic arterial pressure or cardiac output. This decrease was greater under hypoxic conditions when there was increased vascular tone. The mean cardiac output in that study (23) was 6.53 $liters \cdot min^{-1}$ and the estimated pulmonary artery blood concentration of acetylcholine was 4.3×10^{-7} *M*. This level is within the range of estimated pulmonary artery concentrations of acetyicholine at which we observed decreases in pulmonary vascular resistance, although it should be noted that the maximal decrease in pulmonary vascular resistance was only 21.5%. Similarly, in another study (24) of patients with pulmonary hypertension secondary to mitral stenosis, pulmonary artery pressure and pulmonary vascular resistance decreased in seven of nine patients studied, unassociated with any change in cardiac output after bolus injection of acetylcholine. However, in secondary pulmonary hypertension, it has been shown (25) that patients with a higher mean pulmonary artery pressure (for example, >80 mm Hg) tend not to respond to acetylcholine infusion.

There is indirect evidence of a role for endothelium-derived relaxing factor in the regulation of pulmonary vascular tone. In the intact rabbit lung preparation in which vascular tone had been elevated above the baseline and cyclooxygenase activity blocked, acetylcholine infusion (3×10^{-9} to 1×10^{-6} *M*) produced dose-dependent vasodilation (26). Infusion, before and during acetylcholine, of either quinacrine or hemoglobin (both agents antagonize the endothelium-derived relaxing factor-releasing effect of acetylcholine) resulted in further vasoconstriction, implying endothelium-dependent vasodilation in the pulmonary vascular bed. Furthermore, in isolated porcine pulmonary artery rings, this endothelium-dependent vasodilation (in rings preconstricted by phenylephrine) by acetylcholine was greater in small (2 to 3 mm) than in large (5 to 7 mm) arteries (27). This heterogeneity in response may be due to increased receptor density or enhanced efficiency of receptor coupling. To what degree this endothelium-dependent vasodilation determines pulmonary vascular tone and pulmonary blood flow under normal circumstances in not known.

Endothelial cell dysfunction in pulmonary hypertension. Abnormalities of endothelial cells in pulmonary hypertension have been discussed by several groups (28–31). Histologic evidence of endothelial cell injury has been shown (28) to occur early before the development of both pulmonary hypertension and increased vascular reactivity (to hypoxia and epinephrine) after the administration of monocrotaline to rats. More than 10 years ago, Sole et al. (29) observed that the uptake of norepinephrine by the lungs in patients with pulmonary hypertension was reduced and suggested that this reduction was due to endothelial cell dysfunction. Rabinovitch et al. (30) also demonstrated intense immunofluorescent staining for von Willebrand factor in pulmonary arteriolar endothelium of patients with pulmonary hypertension secondary to congenital heart disease and suggested that it is due to altered endothelial cell metabolism. More recently, Dinh Xuan et al. (31) showed impaired endothelium-dependent relaxation in response to acetylcholine in pulmonary vascular rings obtained from patients with pulmonary hypertension secondary to cystic fibrosis.

Several studies (31–35) have reported vasodilation of the pulmonary vascular bed in patients with primary pulmonary hypertension during intravenous infusion of prostacyclin, which causes vasodilation through a direct action on smooth muscle cells (36). Prostacyclin is produced by the pulmonary vascular endothelial cells and because its infusion causes a decrease in pulmonary vascular resistance in these patients, it has been proposed that there may be a deficiency of endothelial prostacyclin production in some forms of pulmonary hypertension. In one comparative study (34), the calcium channel blocking agent nifedipine given orally was as effective in reducing pulmonary vascular resistance as was intracardiac prostacyclin in the same patients, indicating that the increased pulmonary vascular resistance was reversible to a similar degree by two agents with different modes of action on smooth muscle.

Long-term vasodilator therapy. Short-term drug testing has been widely used as a means of predicting the benefit from long-term vasodilator therapy in patients with primary pulmonary hypertension, an abrupt decrease in pulmonary artery pressure or pulmonary vascular resistance indicating a likely long-term effect with subsequent oral vasodilator treatment (9,19,34). The rationale for such testing is that some patients may have a vasoconstrictive component to the increased pulmonary artery pressure that, if reversible during short-term testing, might benefit from long-term therapy with drugs such as nifedipine, diltiazem or hydralazine (7–10). Such an approach may minimize the risk of treating those patients unlikely to respond (9,37,38). Because spontaneous variations in the pulmonary vascular resistance can occur in patients with primary pulmonary hypertension, it was recently suggested (19) that a decrease in pulmonary

vascular resistance should not be considered significant unless it is >30% from baseline. If this criterion is used, up to 50% of patients whose pulmonary vascular resistance decreases during acute drug testing may respond to long-term vasodilation compared with only 6% of those with a reduction in pulmonary vascular resistance of <30% (9). Because no randomized controlled long-term vasodilator studies have been possible, long-term treatment with drugs such as the calcium channel blockers or hydralazine (7,8) in patients who have had such a short-term decrease in pulmonary vascular resistance has not been shown to improve survival (7). It has been suggested (39) that the long-term prognosis relates more to the intrinsic characteristics of the diseased pulmonary vascular bed than to the treatment given. It is possible that dilation of all remaining muscular pulmonary arteries still capable of dilating will help to preserve function in the lung, prevent deterioration of the right ventricle and aid survival in those patients who show a favorable response.

Conclusions. This study has shown that oral nicardipine reduced pulmonary vascular resistance in five patients with primary pulmonary hypertension, indicating that there is a reversible component, although the magnitude of the response was variable. Acetylcholine, calcitonin gene-related peptide and substance P (in two patients) did not change pulmonary vascular resistance, indicating that endothelium-mediated vasodilation does not occur in the pulmonary vascular bed in these patients.

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References

1. Rich S. Primary pulmonary hypertension. Prog Cardiovasc Dis 1988;31:205–38.
2. Dresdale DT, Schultz M, Michtom RJ. Primary pulmonary hypertension. I. Clinical and hemodynamic study. Am J Med 1951;11:686–705.
3. Wood P. Pulmonary hypertension with special reference to the vasoconstrictive factor. Br Heart J 1958;20:557–70.
4. Wagenvoort CA, Wagenvoort N. Primary pulmonary hypertension: a pathologic study of the lung vessels in 156 clinically diagnosed cases. Circulation 1970;42:1163–84.
5. Heath D, Edwards JE. The pathology of hypertensive pulmonary vascular disease: a description of six grades of structural changes in the pulmonary arteries with special references to congenital cardiac septal defects. Circulation 1958;18:533–47.
6. McGoon MD, Vlietstra RE. Vasodilator therapy for primary pulmonary hypertension. Mayo Clin Proc 1984;59:672–7.
7. Rich S, Brundage B, Levy PS. The effect of vasodilator therapy on the clinical outcome of patients with primary pulmonary hypertension. Circulation 1985;71:1191–6.
8. Packer M. Vasodilator therapy for primary pulmonary hypertension. Ann Intern Med 1985;103:258–70.
9. Reeves JT, Groves BM, Turkevich D. The case for treatment of selected patients with primary pulmonary hypertension. Am Rev Respir Dis 1986;134:342–6.
10. Rich S, Brundage B. High-dose calcium channel-blocking therapy for primary pulmonary hypertension: evidence for long-term reduction in pulmonary arterial pressure and regression of right ventricular hypertrophy. Circulation 1987;76:135–41.
11. Rich S, Brundage B. Pulmonary hypertension: a cellular basis for understanding the pathophysiology and treatment. J Am Coll Cardiol 1989;14:545–50.
12. Newman JH, Ross JC. Primary pulmonary hypertension: a look at the future. J Am Coll Cardiol 1989;14:551–5.
13. Pietra GG, Edwards WD, Kay M, et al. Histopathology of primary pulmonary hypertension: a qualitative and quantitative study of pulmonary blood vessels from 58 patients in the National Heart, Lung, and Blood Institute, Primary Pulmonary Hypertension Registry. Circulation 1989;80:1198–206.
14. Furchgott RF. The role of the endothelium in the responses of vascular smooth muscle to drugs. Ann Rev Pharmacol Toxicol 1984;24:175–97.
15. Brain SD, Williams TJ, Tippins JR, Morris HR, MacIntyre I. Calcitonin gene-related peptide is a potent vasodilator. Nature 1985;313:54–6.
16. Newman CM, Maseri A, Hackett DR, El-Tamimi HM, Davies GJ. Response of angiographically normal and atherosclerotic left anterior descending coronary arteries to acetylcholine. Am J Cardiol 1990;66:1070–6.
17. Ludmer PL, Selwyn AP, Shook TL, et al. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med 1986;315:1046–51.
18. Rich S, D'Alonzo GE, Dantzker DR, Levy PS. Magnitude and implications of spontaneous hemodynamic variability in primary pulmonary hypertension. Am J Cardiol 1985;55:159–63.
19. Palevsky HI, Schloo BL, Pietra GG, et al. Primary pulmonary hypertension: vascular structure, morphometry, and responsiveness to vasodilator agents. Circulation 1989;80:1207–21.
20. Clarke B, Grant D, Patmore L, Whiting RL. Comparative calcium entry blocking properties of nicardipine, nifedipine, and PY 108068 on cardiac and vascular smooth muscle (abstr). Br J Pharmacol 1983;78(suppl):333P.
21. Seki T, Takenaka T. Pharmacological evaluation of YC-93, a new vasodilator, in healthy volunteers. Int J Clin Pharmacol 1977;15:267–74.
22. Sorkin EM, Clissold SP. Nicardipine: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy, in the treatment of angina pectoris, hypertension and related cardiovascular disorders. Drugs 1987;33:296–345.
23. Fritts HW, Harris P, Clauss RH, Odell JE, Cournand A. The effect of acetylcholine on the human pulmonary circulation under normal and hypoxic conditions. J Clin Invest 1958;37:99–110.
24. Wood P, Besterman EM, Towers MK, McIlroy MB. The effect of acetylcholine on pulmonary vascular resistance and left atrial pressure in mitral stenosis. Br Heart J 1957;19:279–86.
25. Harris P. Influence of acetylcholine on the pulmonary arterial pressure. Br Heart J 1957;19:272–8.
26. Cherry PD, Gillis CN. Evidence for the role of endothelium-derived relaxing factor in acetylcholine-induced vasodilatation in the intact lung. J Pharmacol Exp Ther 1987;241:516–20.
27. Zellers TM, Vanhoutte PM. Heterogeneity of endothelium-dependent and independent responses among large and small porcine pulmonary arteries. Pulmon Pharmacol 1989;2:201–8.
28. Rosenberg HC, Rabinovitch M. Endothelial injury and vascular reactivity in monocrotaline pulmonary hypertension. Am J Physiol 1988;255:H1484–91.
29. Sole M, Drobac M, Schwartz L, Hussain MN, Vaughan-Neil EF. The extraction of circulating catecholamines by the lungs in normal man and in patients with pulmonary hypertension. Circulation 1979;60:160–3.
30. Rabinowitch M, Andrew M, Thom H, et al. Abnormal endothelial Factor VIII associated with pulmonary hypertension and congenital heart defects. Circulation 1987;76:1043–52.
31. Dinh Xuan AT, Higenbottam TW, Pepke-Zuba J, Chellaoui C, Wallwork J. Reduced endothelium-dependent relaxation of cystic fibrosis pulmonary arteries. Eur J Pharmacol 1989;163:401–3.
32. Rubin LJ, Groves BM, Reeves JT, Frosolono M, Handel F, Cato AE. Prostacyclin-induced acute pulmonary vasodilation in primary pulmonary hypertension. Circulation 1982;66:334–8.
33. Jones DK, Higenbottam TW, Wallwork J. Long-term treatment for primary pulmonary hypertension with intravenous prostacyclin. Br Heart J 1987;57:270–8.
34. Roskovec A, Stradling JR, Shepherd G, MacDermott J, Oakley CM, Dollery CT. Prediction of favourable responses to long-term vasodilator

treatment of pulmonary hypertension by short-term administration of epoprostenol (prostacyclin) or nifedipine. Br Heart J 1988;59:696–705.

35. Rubin LJ, Mendoza J, Hood M, et al. Treatment of primary pulmonary hypertension with continuous intravenous prostacyclin (epoprostenol). Ann Intern Med 1990;112:485–91.

36. Botting R, Vane JR. Vasoactive mediators derived from the endothelium. Arch Mal Coeur 1989;82:9–95[1]:11–4.

37. Packer M, Greenberg B, Massie B, Dash H. Deleterious effects of sodium nitroprusside in patients with primary pulmonary hypertension. N Engl J Med 1987;316:1370–31.

38. Packer M, Medina N, Yushak M. Adverse hemodynamic and clinical effects of calcium channel blockade in pulmonary hypertension secondary to obliterative pulmonary vascular disease. J Am Coll Cardiol 1984;4:890–901.

39. Dunkler DR, D'Alonzo GE, Gimotti L, Fuentes F, Nickerson D, Fracasso M. Vasodilators and primary pulmonary hypertension: a weighted life-long-term response. Chest 1989;95:1385–9.